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DNA end resection: nucleases team up with the right partners to initiate homologous recombination

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Abstract

The repair of DNA double-strand breaks (DSBs) by homologous recombination commences by nucleolytic degradation of the 5'-terminated strand of the DNA break. This leads to the formation of 3'-tailed DNA, which serves as a substrate for the strand exchange protein Rad51. The nucleoprotein filament then invades homologous DNA to drive template-directed repair. In this review, I discuss mainly the mechanisms of DNA end resection in *Saccharomyces cerevisiae*, which includes short-range resection by Mre11-Rad50-Xrs2 and Sae2, as well as processive long-range resection by Sgs1-Dna2 or Exo1 pathways. Resection mechanisms are highly conserved between yeast and humans, and analogous machineries are found in prokaryotes as well.

Introduction

Homologous recombination (HR) plays a central role in the repair of DNA double-strand breaks (DSBs) (1). In vegetative cells, recombination restores broken DNA to preserve genome integrity. In meiosis, HR promotes proper chromosome segregation and exchange of genetic information between maternal and paternal genomes, and thus contributes to generation of genetic diversity. Recombination is initiated upon the formation of single-stranded DNA (ssDNA) overhangs through a process termed DNA end resection. The nucleolytic processing of broken DNA ends is essential for all recombination mechanisms (Fig. 1). Resection of

DSBs commits their repair to HR as it prevents ligation by the potentially more mutagenic non-homologous end-joining (NHEJ) pathway (2-4). Resected DNA is first coated by the ssDNA binding protein Replication Protein A (RPA). In most cases, RPA is subsequently replaced with the strand exchange protein Rad51, forming a nucleoprotein filament capable to invade homologous DNA. Repair can then proceed *via* either of two main recombination pathways, synthesis-dependent strand annealing (SDSA) or the canonical pathway that involves the formation of a double Holliday junction (DSBR) (Fig. 1). Single-strand annealing (SSA) is instead a Rad51-independent pathway that requires extensive resection of DNA between two repetitive sequences (Fig. 1).

1. DNA end resection: when and what to resect

DSBs can form accidentally in any phase of the cell cycle upon exposure to ionizing radiation, chemicals or as a result of abortive processing of nucleic acids. Most DSBs however occur in S-phase when a DNA replication fork runs into a nick. Furthermore, DSBs are sometimes introduced "intentionally" such in the prophase of the first meiotic division or during anti-cancer therapy regimens based on DNA damaging agents (5). Depending on the cellular context, cells must first "decide" whether or not to resect the breaks (3,4,6,7). DNA end resection commits the repair to HR and prevents NHEJ; therefore, it would be detrimental to resect DSBs in the G1 phase of the cell cycle when no sister chromatid DNA is available

as a template for repair. Cells have thus developed regulatory control mechanisms that activate resection only during the S or G2 phases of the cell cycle, which will be introduced below (4,6-8).

Another critical parameter is the polarity of resection. It has been observed *in vivo* that the 5'-terminated strand of the dsDNA break is specifically resected (9,10). Although limited processing of the 3'-terminated strand has been observed as well (11), the preferential degradation of the 5'-terminated strand results in the formation of 3'-tailed DNA. This becomes a substrate for Rad51 and upon strand invasion the 3' end primes DNA synthesis, which is required for the downstream steps in the HR pathway (1). How the various DNA end processing machineries ensure the 5'→3' polarity of resection will be discussed.

2. DNA end resection: lessons from prokaryotes

In *Escherichia coli*, DNA end resection is carried out either by the RecBCD- or RecQJ-dependent pathways (12). RecBCD is a vigorous nuclease-helicase complex with a strong affinity towards DNA ends. RecB has a helicase activity that unwinds DNA in a 3'→5' direction, which functions synergistically with the RecD helicase subunit. RecD translocates on the opposite strand than RecB with a 5'→3' polarity resulting in a net translocation in the same direction away from the DNA end (13-15). The RecB and RecD motors do not run at the same speed. The unique bidirectional translocation mechanism gives rise to a ssDNA loop that accumulates in front of the slower RecB subunit, and which is detectable by electron microscopy (15). Before encountering the regulatory Chi (crossover hotspot instigator) sequence within genomic DNA, the RecB subunit degrades both 5' and 3'-terminated DNA strands. Upon encountering Chi, the complex pauses and continues translocating at a reduced speed dependent on RecB, which becomes the lead motor (13). Importantly, the nucleolytic degradation of the 5'-terminated DNA is upregulated, while the degradation of the 3'-terminated strand is attenuated, which determines the polarity of DNA end resection (16). DNA without a Chi sequence is fully

degraded by RecBCD, which contributes to prokaryotic defense mechanisms against invading DNA. Furthermore, RecBCD directly loads the strand exchange protein RecA on the arising 3'-tailed DNA, which facilitates recombination (17).

The RecQJ enzymes initiate a second major recombination pathway in *E. coli*, which also requires the RecFOR factors (12). RecQ, a founding member of the RecQ helicase family, unwinds dsDNA with a 3'→5' polarity, which generates ssDNA for the RecJ nuclease that degrades DNA 5'→3' in a manner stimulated by the ssDNA binding protein SSB (12,18). Therefore, unlike RecBCD, the activity of the RecQJ complex directly produces 3'-tailed DNA and the resection polarity is not regulated by the Chi sequence. As RecBCD, the RecFOR complex also loads RecA on the SSB-coated ssDNA at junctions of single and double-stranded DNA (19).

While the RecFOR pathway is conserved across prokaryotes, the RecBCD complex is only present in some bacteria that include gram-negative *E. coli* (20). In gram-positive *Bacillus subtilis*, RecBCD is replaced by the AddAB complex (20). AddAB has a single motor within the AddA subunit that unwinds DNA with a 3'→5' polarity, which is stimulated by the AddB subunit (21). While no Chi sequence has been detected in eukaryotes, variations of similar helicase-nuclease complexes that resect DSBs are conserved in evolution.

3. Two-step resection model: the relationship between short and long-range resection pathways

DNA end resection in eukaryotes is a two-step process in most cases (9,22,23). It is initiated by a nucleolytic processing step that is slow and limited to the vicinity of DNA ends (9,23). In *S. cerevisiae*, this first step is dependent on the Mre11-Rad50-Xrs2 (MRX) complex. MRX has an affinity for DNA ends, and was shown to be one of the first proteins recruited to DSBs (24,25). It has both catalytic and structural roles in DNA end processing. The intrinsic nuclease activity of Mre11 is capable to degrade 5'-terminated DNA in the vicinity of the DNA end. The structural role of MRX involves recruitment of components belonging to the second long-range processing step (9,23,26-

29). In yeast, these include two separate pathways dependent on either the Sgs1-Dna2 helicase-nuclease or the Exo1 nuclease (Fig. 2).

DSBs arise in multiple ways and thus are very diverse in structure (5). Some are chemically "clean" and may either be blunt-ended or have short 5' or 3' ssDNA overhangs. These stretches of ssDNA may form secondary structures that impede resection. Many DSBs are chemically "dirty", including those induced by ionizing radiation, which in addition to DNA breakage gives rise to oxidative DNA damage. Furthermore, DSBs can arise upon abortive topoisomerase reactions that may occur either spontaneously or upon drug treatment. E.g. the anti-cancer drug etoposide inhibits Topo II, which remains trapped at the 5'-terminated strand of the DSB (30). Finally, DSBs in meiosis are introduced by the Topo II-like enzyme Spo11, which also remains covalently attached to the 5' end of the broken DNA (31-33). The presence of secondary structures, chemical modifications or proteins at the DNA end represents a specific challenge to the resection machinery. It has been demonstrated that the short-range resection pathway, and specifically the nuclease of Mre11, is required for the processing of these non-canonical DNA ends (26,34,35). The Mre11 nuclease activity is instead largely dispensable for the resection of endonuclease-induced "clean" DSBs (36) (Fig. 2). Similarly, the structural role of MRX is not essential, as Exo1 and Sgs1-Dna2 can initiate resection of clean DNA ends in MRX-independent manner however less efficiently (9,27-29,37).

Long-range resection pathways were initially identified using physical assays that measure the kinetics of ssDNA formation at various distances from an experimentally induced dsDNA break (9,23). In order to improve detection, these assays were performed in a *rad51Δ* background that does not allow the repair of the DSB. In addition, genetic assays based on SSA were utilized, in which long tracts of DNA must be resected to reveal a repeated sequence to allow repair (9,23). Together, these assays showed that Sgs1-Dna2 or Exo1 pathways are capable to resect very long stretches of DNA of more than 50,000 nts in length (9).

Subsequent work revealed that these assays largely overestimated the length of DNA that is resected *in vivo* under normal conditions when repair is possible. In mitotic cells, it has been determined that ~2,000-4,000 nts are resected in allelic recombination and ~3,000-6,000 nts in ectopic recombination (38). In meiotic cells, where the long-range resection is largely dependent on Exo1, the resection tracks are even shorter (~800 nts) (39). In the *sgs1Δ exo1Δ* double mutant that is deficient in long-range resection, the degradation tracks are reduced to ~100-300 nts in mitotic cells and ~270 nts in meiotic cells (38,39). Intriguingly, the limited MRX and Sae2 dependent resection is sufficient for efficient joint molecule formation in meiosis, and results in only a moderate recombination defect in vegetative cells (30-50% reduction)(9,38). Therefore, long-range resection is largely dispensable for recombination in meiosis, and not strictly required for repair in vegetative cells, although it may be necessary for proper DNA damage checkpoint and maintenance. In gene targeting, elimination of the long-range resection pathways increased efficiency up to 600-fold (38). This demonstrated that Sgs1-Dna2 or Exo1 over-resected the transformed DNA. The short-range processing by MRX-Sae2 complex was sufficient for homology search and repair (38).

4. Short-range DNA end processing by MRX and Sae2: mechanism and regulation

The MRX complex likely functions as a dimer (40,41). It has a DNA binding activity with a preference towards DNA ends (24,42). The Rad50 subunit is an ATPase that controls conformation changes within the complex upon DNA binding, which regulates its functions in DNA end tethering, resection and DNA damage signaling (43-45). *In vitro*, Mre11 is a manganese-dependent exonuclease that is moderately stimulated by Xrs2 (24). Mre11 also has a much weaker endonuclease activity on diverse secondary structures that is moderately promoted by Rad50 in the presence of ATP (46). However, the polarity of the Mre11 exonuclease (3'→5') was in disagreement with the polarity of resection observed *in vivo* (5'→3') as

well as with the DSB repair model that postulates that 3'-tailed ssDNA tails must be generated (46-48). To this point, it was shown that *P. furiosus* Mre11-Rad50 has a weak magnesium-dependent endonuclease activity on the 5'-terminated strand near a DNA end (49). Later, it was demonstrated with recombinant *S. cerevisiae* proteins that Sae2 strongly promotes the endonuclease of Mre11 within the MRX complex (50). Similarly as in the Hopkins and Paull study (49), the endonuclease activity was magnesium-dependent and showed a preference towards 5'-terminated DNA. The preferential cleavage of the 5'-terminated DNA ~15-25 nucleotides away from the end suggested that the Mre11 nuclease initiates DNA resection *via* its endonuclease, rather than exonuclease activity. Furthermore, the endonucleolytic 5' end clipping was strongly promoted by protein blocks at the DNA end, demonstrating a possible mechanism of processing non-canonical DNA ends that are refractory to exonucleases (50). Under physiological conditions, when magnesium concentrations strongly exceed those of manganese and when DNA ends are protected by a number of factors, the Mre11 exonuclease activity might be attenuated and MRX might preferentially function as a Sae2-promoted endonuclease (50).

The biochemical reconstitution experiments validated models that have been for a long time inferred from genetic studies. Specifically in meiosis, the Spo11 protein was found in complexes with oligonucleotide DNA molecules of ~12 and ~21-37 nucleotides in length (31,51). These DNA fragments were attached to Spo11 *via* their 5' end and had a free 3' DNA end, which suggested that the processing of meiotic DSBs is initiated by an endonucleolytic cut. The MRX complex was proposed as being the best candidate for the enigmatic nuclease. Subsequent studies revealed that end processing, at least in some cases, is initiated by a cut at a position more distant from the DNA end, up to ~100-300 nucleotides away (52). This collectively provided support for a bidirectional resection model, which posits that upon the initial endonuclease cleavage, the Mre11 exonuclease proceeds back towards the DNA end *via* its 3'→5' exonuclease activity (Fig. 2). At the

same time, the endonuclease cut can create an entry point for the long-range resection enzymes. However, on the mechanistic level, it remains to be determined how the endonucleolytic cleavage by Mre11 is directed to the more distant sites away from the DNA break.

Genetic experiments also revealed that the Sae2 protein functionally integrates with the MRX complex (32). The phenotypes of *sae2Δ* cells resemble those of *mre11* nuclease-deficient mutants in many genetic assays. In meiosis, *sae2Δ* strains are completely deficient in the processing of Spo11-bound DNA breaks; furthermore, *sae2Δ* also affects Mre11 nuclease function in mitotic cells (32,53-56). This led to the notion that Sae2 might activate the nuclease of Mre11, as later directly demonstrated by reconstitution experiments (50). In contrast, cells lacking *SAE2* are more sensitive than *mre11* nuclease-dead mutants to DNA damaging agents (26). Thus, in addition to stimulating the Mre11 endonuclease, Sae2 has other, Mre11-nuclease independent roles. This may include its proposed function to remove MRX from DNA ends upon end processing to facilitate downstream repair, attenuate checkpoint signaling, counteract the NHEJ factor Ku and promote resection by Exo1 (26,29,57-59). Sae2 itself was also shown to possess a nuclease activity specific to secondary structures in DNA (60), although an enzymatic activity was not detected by other laboratories (27,50). Human and *S. pombe* Sae2 homologues (CtIP and Ctp1, respectively) were found to tetramerize, which was shown to be important for their function *in vivo* (61,62). Similarly, mutations that prevent oligomerization of Sae2 *in vivo* resulted in null phenotypes in several genetic assays (53). Intriguingly the nuclease of Sae2 has been suggested to be specific to its monomeric form (63). Taking together, the role of Sae2 in DNA metabolism is still only partially defined.

The Sae2 function in regulating the nuclease of Mre11 makes it an ideal target for control by posttranslational modifications (8). Indeed, Sae2 is phosphorylated in S/G2 phases of the cell cycle by the cyclin-dependent protein kinase (CDK) Cdc28 (4,6). The key CDK target site is likely S267, which must undergo phosphorylation to

allow resection both *in vivo* and *in vitro* (6,50). The phosphomimicking mutant Sae2 S267E partially rescues resection defect in the absence of CDK activity, while the non-phosphorylatable S267A mutant phenotype is comparable to that of *sae2Δ* cells (6). Therefore, the CDK-dependent regulation of Sae2 activity represents one of the key control mechanisms ensuring that resection only takes place in the S/G2 phase of the cell cycle when a homologous template is available for repair. In addition to CDK, Sae2 is also regulated by the Mec1 and Tel1 kinases in response to DNA damage (63-65). Phosphorylation of Sae2 was shown to affect its oligomeric state (63). Furthermore, mutations of Mec1/Tel1 target sites to non-phosphorylatable residues in Sae2 result in DNA damage sensitivity, showing that also phosphorylation under the control of DNA damage checkpoint is important for the function of Sae2 *in vivo* (63-65). As Sae2 has additional roles on top of controlling Mre11 (see above), it remains to be determined whether the Mec1/Tel1-dependent phosphorylation affects DSB resection or other functions of Sae2.

In higher eukaryotes, the homologue of MRX is the MRN complex, which consists of MRE11, RAD50 and NBS1 subunits (66,67). Similarly as in yeast, recombinant MRN has a manganese-dependent 3'→5' exonuclease and a weaker endonuclease activity (47,48,68). The human counterpart of Sae2 is CtIP, though the sequence homology is restricted to its C-terminal part as CtIP is a much larger protein than Sae2 (69). Experiments based on small molecule inhibitors that target specifically the endonuclease or the exonuclease of human MRE11 revealed that the endonuclease precedes the exonuclease in resection (2). Thus, the bidirectional resection is likely conserved in evolution and not limited to meiosis. However, whether and how CtIP regulates the MRE11 endonuclease has not been directly established yet. In contrast to yeast however, the activity of MRN and CtIP in DNA end resection cannot be bypassed, as DNA end resection is generally dependent on the presence of CtIP and the nuclease activity of MRE11 (69).

5. Long-range DNA end processing by Sgs1-Dna2 or Exo1

While the involvement of MRX in the processing of DNA ends has been known for a long time (70), the pathways responsible for the long-range resection were identified much later. This is most likely due to that fact that long-range resection can be carried out by either of two non-overlapping pathways, dependent on the enzymatic activities of Sgs1-Dna2 or Exo1 (9,22,23). Inactivation of a single pathway results in only a minor resection defect, because the other pathway can effectively compensate. Major resection defects were only revealed when both pathways were inactivated simultaneously, e.g. in *sgs1Δ exo1Δ* double mutants (9,22,23).

5.1. Sgs1-Dna2 resection pathway

Both Sgs1 and Dna2 have separate functions unrelated to DNA end resection. Sgs1 is a vigorous DNA helicase belonging to the RecQ family (71,72), which functions together with Top3 and Rmi1 to dissolve double Holliday junctions into non-crossover products, thereby preventing sister-chromatid exchanges and chromosome instability (73,74). Dna2 is a bifunctional helicase-nuclease responsible for removing DNA flaps arising by strand displacement synthesis by DNA polymerase δ during lagging strand DNA synthesis (75). The Okazaki fragment processing function of Dna2 is essential, although the viability of *dna2Δ* mutants can be rescued by multiple mechanisms (76). Prior to the seminal work by Ira and colleagues (9), Sgs1 and Dna2 had not been implicated to function together.

The mechanism of DNA end resection by the Sgs1-Dna2 pathway was revealed by a combination of genetic and biochemical experiments. The helicase of Sgs1 unwinds dsDNA with a 3'→5' polarity, which provides a substrate for the ssDNA-specific Dna2 nuclease (9,27,28). Dna2 must load on a free ssDNA end but then degrades DNA endonucleolytically resulting in degradation products of ~5-10 nucleotides in length (77). Dna2 was shown to possess both 3'→5' and 5'→3' nuclease activities (78), so its involvement in DNA end resection was initially puzzling. The issue was resolved later when it was demonstrated that RPA inhibits

the degradation of 3'-terminated ssDNA, while it stimulates the degradation of the 5'-terminated strand (27,28). Therefore, RPA is a crucial factor that enforces the correct polarity of DNA end resection by the Sgs1-Dna2 pathway, leading to the production of 3'-tailed DNA (Fig. 3a).

Dna2 also possesses a DNA helicase activity with a 5'→3' polarity. Unlike the nuclease of Dna2 that is essential for cell viability, helicase-deficient *dna2* mutants are viable under certain growth conditions (76). The physiological role of the Dna2 helicase is not yet clear. The DNA unwinding activity of Dna2 is vigorous, comparable to the helicase capacity of Sgs1, yet it is cryptic and only becomes apparent upon inactivation of the Dna2 nuclease (79). It is tempting to think that the helicase of Dna2 functions in concert with that of Sgs1 (28). Both Sgs1 and Dna2 were shown to directly interact, which led to the model where Sgs1 translocates along one DNA strand in a 3'→5' direction and unwinds DNA, whereas Dna2 translocates with a 5'→3' polarity on the second DNA strand unwound by Sgs1, yet in the same general direction as Sgs1 (28). This mode of translocation and DNA degradation would be reminiscent of the resection complexes from bacteria such as RecBCD (14); though, it has not been substantiated biochemically. Specifically, in contrast to a bidirectional manner of DNA translocation by Sgs1-Dna2, the helicase activity of Dna2 was implied to be dispensable for DNA end resection (9). Similarly to the nuclease domain of *B. subtilis* AddA, also Dna2 contains a 4Fe-4S iron-sulfur cluster that appears to have a structural role, which further highlights the parallels between prokaryotic and eukaryotic resection complexes (21). More experiments are clearly needed to determine whether and how the helicase of Dna2 within the Sgs1-Dna2 heterodimeric complex promotes resection.

Several factors have been identified that stimulate DNA end resection by Sgs1-Dna2, which includes the MRX complex and the Top3-Rmi1 heterodimer (9,27,28). As discussed above, the nuclease of Mre11 is largely dispensable for the processing of clean DSBs, yet MRX was shown to have a structural role in promoting the resection by Sgs1-Dna2. In particular, Mre11 interacts

with Sgs1 and stimulates its helicase activity (27,28,80). As the MRX complex localizes very early to DSBs (25), it has been proposed that it might recruit Sgs1-Dna2 to DNA ends (81). Furthermore, the Sgs1 helicase is known to form a complex with Top3-Rmi1 (72,82). Surprisingly both Top3 and Rmi1 were found to be required for DNA end resection by Sgs1 and Dna2 *in vivo* (9) as well as *in vitro*, independently of the topoisomerase activity of Top3 (27). The heterodimer strongly stimulates the Sgs1 helicase, which is especially apparent under physiological salt concentrations (27,28). The mechanism by which Top3-Rmi1 promote DNA unwinding by Sgs1 is not yet clear, though it is obvious that Sgs1-Top3-Rmi1 form a very integrated functional complex (82,83). Additionally, Sgs1 was described to interact with Rad51 (84). The functional significance of this interaction is not yet clear, however it is attractive to hypothesize that it might help loading Rad51 directly on resected ssDNA in analogous fashion to RecBCD- or RecFOR-mediated loading of RecA (17).

The mechanism of DNA end resection by Sgs1 and Dna2 is conserved in evolution. Human DNA2 forms a complex with the human Sgs1 homolog, the Bloom (BLM) helicase, and the resection by DNA2-BLM is similarly promoted by the human RPA, MRN and TopoIIIα-RMI1-RMI2 proteins (85,86). In addition, DNA2 also interacts with another RecQ family helicase, Werner (WRN). Also BLM-WRN complex promotes resection *in vivo* and *in vitro*, showing a functional redundancy in DSB processing in human cells (87).

5.2. Exo1 resection pathway

Unlike the Dna2 nuclease that is specific for ssDNA, the nuclease activity of exonuclease 1 (Exo1) degrades 5'-terminated strand within dsDNA (88). Therefore, Exo1 does not require a helicase partner to unwind DNA, and directly produces the required 3'-tailed DNA (37,88) (Fig. 3b). In humans, the BLM protein was found to stimulate resection by EXO1 in a helicase-independent manner, but a similar mechanism was not detected in yeast (9,22,23,37,89,90).

Before Exo1's role in DNA end resection was discovered, Exo1 had been known to play an important function in the postreplicative mismatch repair. Reconstitution experiments revealed that Exo1 nuclease is rather distributive and requires the support of the mismatch recognition complex MutSα to stimulate its processivity in the presence of a mismatch (91). Similarly, various factors were identified that promote the Exo1 nuclease in resection. As in the case of the Sgs1-Dna2 pathway, the MRX complex provides a structural role to stimulate Exo1 (37,81), which is further enhanced by Sae2 (29). However, efficient Exo1-dependent resection occurred even in the absence of the MRX complex *in vivo*, suggesting that other factors may promote the Exo1 nuclease (9,23). That may include the ssDNA binding proteins RPA or the Sensor of ssDNA complex 1 (SOSS1) (37,92,93). Further-

more, the 9-1-1 clamp was also found to promote long-range resection independently of its checkpoint signaling activity under certain conditions (94), which is conserved in human cells (95). Finally, PCNA was found to promote human EXO1 processivity by enhancing its association with DNA (85,96).

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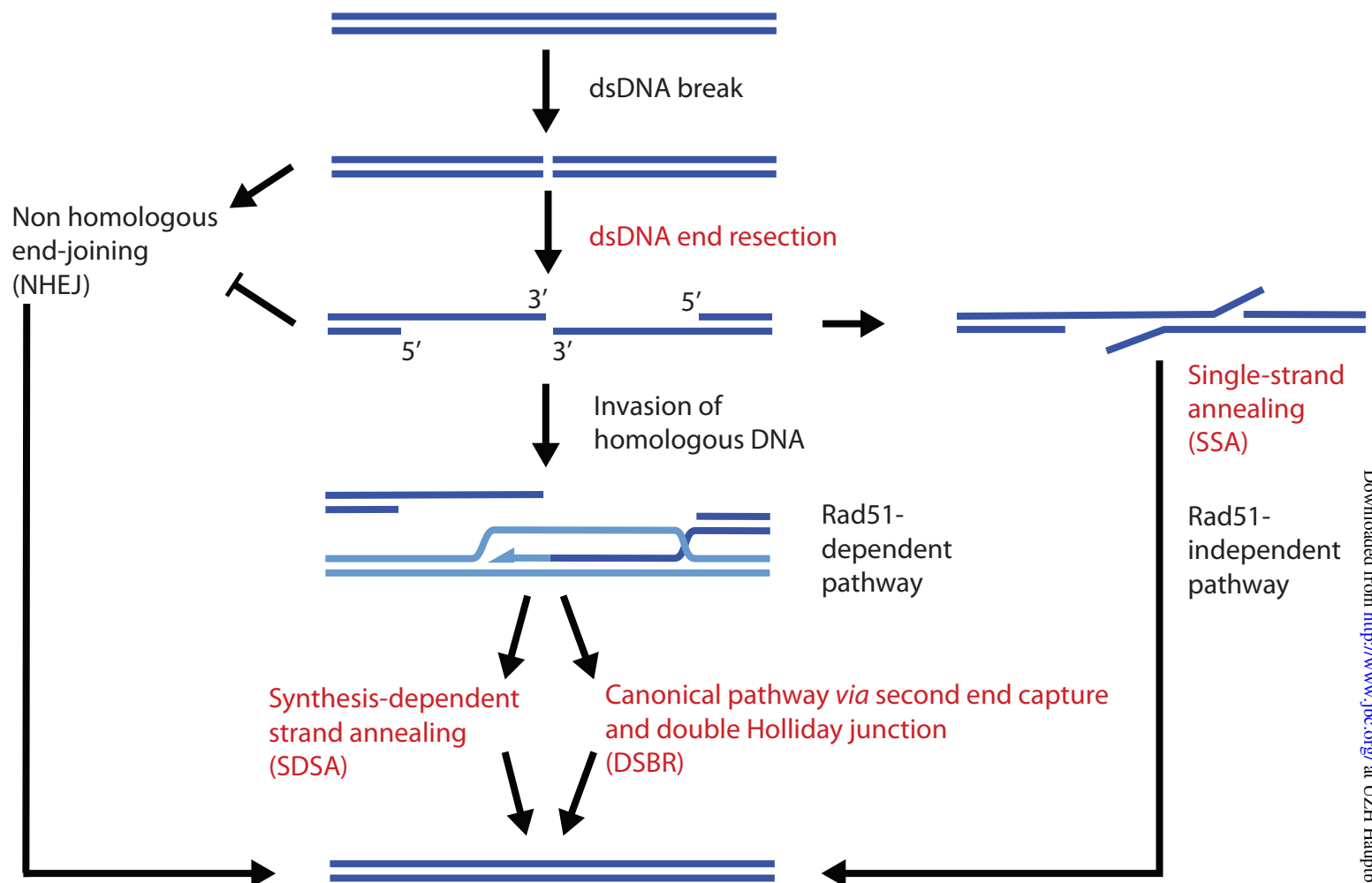
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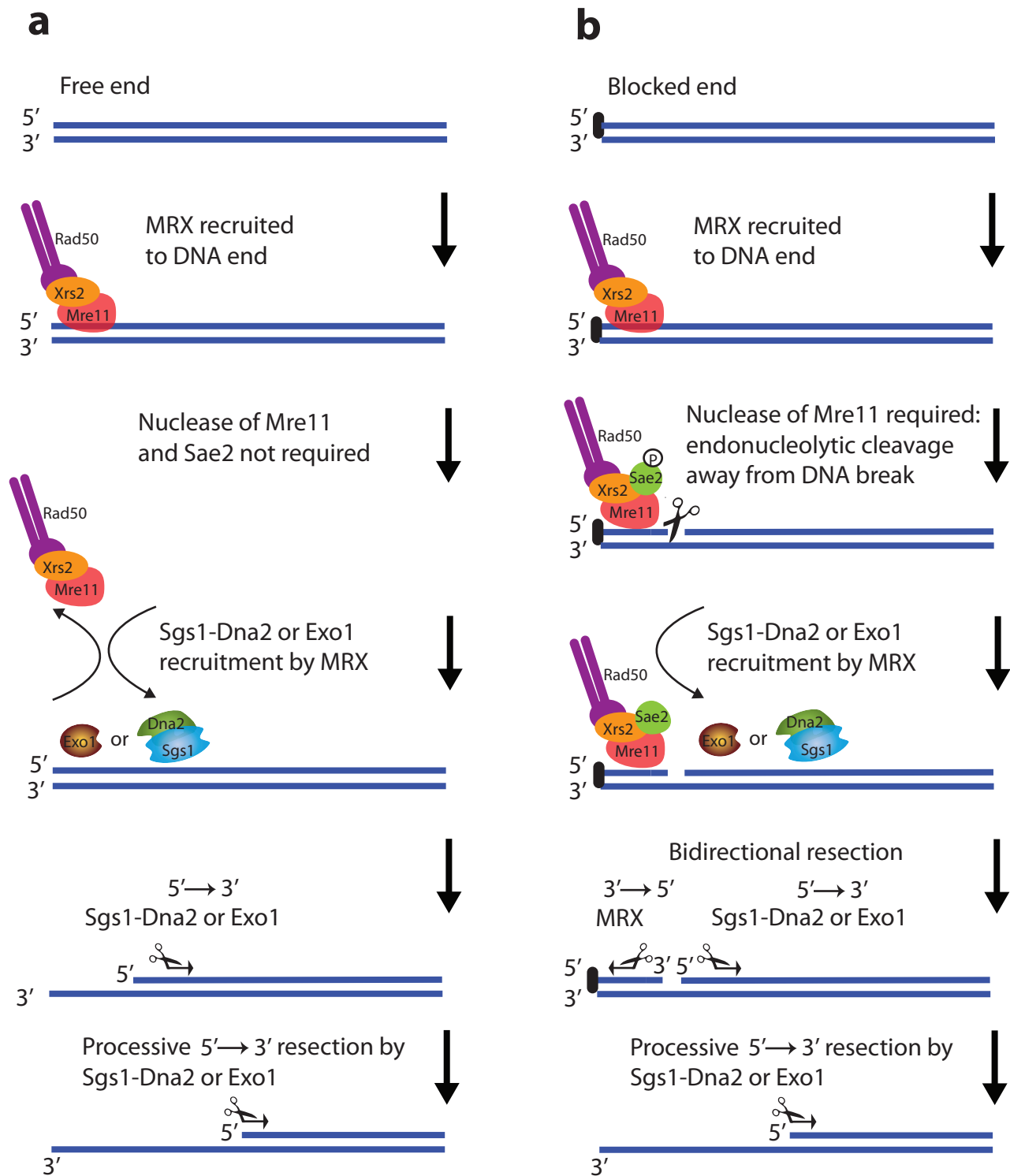
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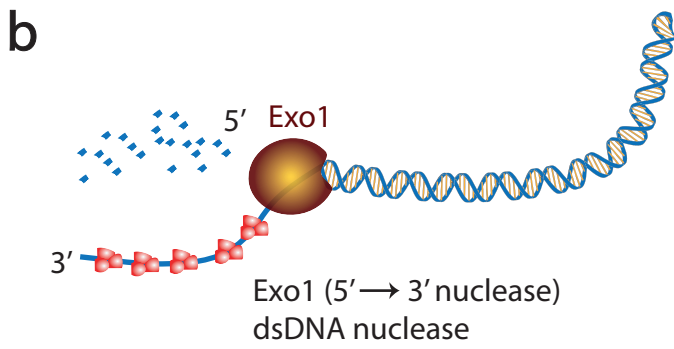
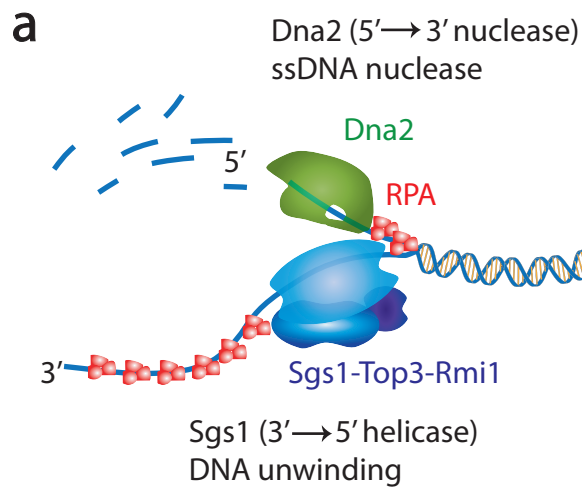
Figure 1. DNA end resection is required for all recombination processes. The resection of the 5'-terminated DNA strand is required for all recombination pathways, including single strand annealing (SSA), synthesis-dependent strand annealing (SDSA) and canonical double strand break repair (DSBR) pathway. DNA end resection prevents mutagenic non-homologous end-joining (NHEJ). Microhomology-mediated end-joining (MMEJ) was omitted from the scheme and text for simplicity.

Figure 2. DNA end resection of free and blocked DNA ends. **a**, Resection of free (clean) DNA ends. The Mre11-Rad50-Xrs2 (MRX) complex is rapidly recruited to DNA ends upon break formation. The nuclease activity of Mre11 is not required for resection, but the MRX complex has a role to recruit components of the processive pathways that include either Sgs1-Dna2 or Exo1. In some cases the structural role of the MRX complex can be bypassed. DNA is subsequently resected by either Sgs1-Dna2 or Exo1 in a processive manner. Only a monomer of MRX is depicted for clarity reasons. **b**, Resection of blocked (dirty) DNA ends. The MRX complex is rapidly recruited to DNA ends, which is followed by Sae2. The nuclease activity of Mre11 is required, and it cleaves endonucleolytically the 5'-terminated DNA strand away from the end in a reaction stimulated by phosphorylated Sae2. Furthermore, MRX also likely recruits Sgs1-Dna2 or Exo1 to the endonuclease cut site. The endonuclease cut site provides an entry point for the Sgs1-Dna2 or Exo1 nucleases, which carry out long-range resection. The exonuclease of Mre11 then might degrade DNA in a 3' → 5' direction back towards the DNA break.

Figure 3. Mechanism of long-range DNA end resection by Sgs1-Dna2 or Exo1 pathways. **a**, DNA end resection by Sgs1-Dna2. Sgs1 translocates with a 3' → 5' polarity on one DNA strand and unwinds DNA. Unwound ssDNA is coated by RPA, which directs the nucleolytic activity of Dna2 towards the 5'-terminated DNA strand. Whether the 5' → 3' motor activity of Dna2 participates in DNA end resection to form a bidirectional helicase remains to be demonstrated. **b**, DNA end resection by Exo1. The Exo1 nuclease is specific for dsDNA and has a 5' → 3' polarity, which directly results in 3' tailed DNA.







Minireview:

**DNA end resection: nucleases team up with
the right partners to initiate homologous
recombination**

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